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Microbiology #3

<u>Comparison of Cell Walls of Gram-Positive and Gram-Negative Bacteria:</u>

- The Peptidoglycan layer is much thicker in gram-positive bacteria than in gram-negative bacteria (*Peptidoglycan structure: is composed of a glycan chain (NAM and NAG), a tetrapeptide chain, and a cross-link (peptide interbridge).

- Only gram-negative bacteria have a complex outer membrane consisting of endotoxin (lipopolysaccharide [LPS]). In addition to proteins which are called porins which facilitate the passage of small, hydrophilic molecules.

- Gram-negative bacteria have a periplasmic space which is found between the outer membrane layer and the cytoplasmic membrane. Periplasmic space is the site for enzymes called β-lactamases that degrade penicillins and other antibiotics (β- lactam drugs)

- Teichoic acids are fibers that are found in the outer layer of several important gram-positive bacteria, such as staphylococcus and streptococcus. They are composed of polymers of either glycerol phosphate or ribitol phosphate linked via phosphodiester bonds (staphylococcus)
  - Teichoic acids are responsible for:
    1. Giving support to cell wall.
    2. Inducing inflammation and septic shock when caused by certain gram positive bacteria.

Gram stain: the basic difference is the structure of the cell wall of the gram negative bacteria**, which stain red** vs. gram positive bacteria **which stain blue.** And it is a differential stain.

The Gram stain involves the following four-steps:

1- The crystal violet dye stains all cells blue/purple.

2- The iodine solution is added to form a crystal violet–iodine complex.

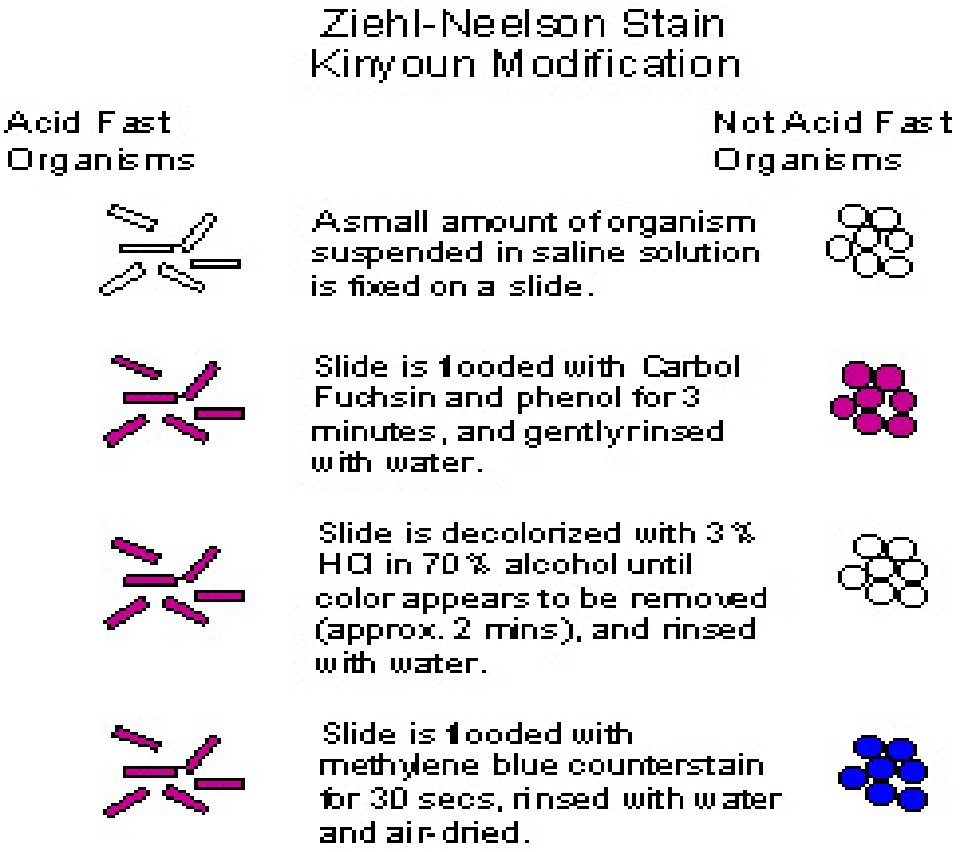
3- Decolonization by acetone or ethanol, extracts the blue dye complex from the lipid-rich, thin-walled gram-negative bacteria to a greater degree than from the lipid-poor, thick-walled gram-positive bacteria.

4- The red dye safranin stains the decolorized gram-negative cells **<u>red/pink; the gram-positive bacteria remain blue.</u>**

* The exceptions for gram staining is that there are some organisms like: Mycobacterium tuberculosis which causes tuberculosis that cannot be Gram-stained because they resist decolonization with acid–alcohol after being stained. This property is related to the high concentration of lipids, called mycolic acids, in the cell wall of mycobacteria.

(This peptidoglycan cell wall doesn't allow to the stain enter easily because it is rich with mycolic acids).

Acid-fast stain is used for this organism and it is a deferential stain.



The primary stain in the acid fast stain is carbol fuchsin and it is **red**. Then we use acid-alcohol (3% HCl and 70% alcohol) which is a strong decolorizer. (red color means acid-fast)

Results: Acid-fast organisms are red, while nonacid-fast are blue.

We have **two groups** from bacteria that are acid-fast stained:

1- Mycobacterium

2- Nocardia species

*** Flagella stain:** is a special stain for other parts.

***Capsule stain:** it's a type of staining**.**

*All bacteria have a cell wall composed of peptidoglycan except for mycoplasma, thus it has no regular shape (pleomorphic). Mycoplasma are also smallest free living organisms (bacteria) (<0.2 µm) which contains all cellular components such as DNA, RNA, …etc. and can divide by binary fission.

NOTE: Most, but not all, Eukaryotic cell membranes contain steroids.

- **Bacteria with deficient cell wall: (L- forms):**

Bacteria include phases where they transform into small forms that lose their cell walls. This means that they can no longer be killed by many commonly used antibiotics. These bacteria are called cell wall deficient (CWD) or L-form bacteria.

NOTE: CWD Bacteria have a genetic material to produce a cell wall but for some reason they stop producing a cell wall.

There are two types of L- form bacteria:

1. Spheroplasts: **Gram negative bacteria with residual cell wall material**
2. Protoplasts: **Gram positive bacteria without cell wall.** Lysozyme-treated bacteria that can survive in isotonic solutions.
   These two terms mean, less cell wall (partial cell wall)

NOTE: Antibiotics can be selective for bacterial cell since they sometimes target the cell wall.
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- Composition of cell membrane:

1. Phospholipid bilayer

* Serum: is the liquid portion of the blood, we prepare it using centrifuge. Serum contains antibodies and other proteins (it is blood plasma without fibrinogens).

-Serotype: we recognize it by using serum.

- **Cytoplasm consists of:**

- **Genome:**

The nucleoid is the area of the cytoplasm in which DNA is located. The DNA of prokaryotes is a single, circular molecule.

- **Plasmid:**

A plasmid is a small, circular, double-stranded DNA molecule that is not essential for bacterial division.

We have two types of plasmid:

1- R plasmid (Resistance plasmid): Resistance and degradation of antibiotics
2- F plasmid (Fertility plasmid)/Conjugated plasmid: Carrying genes for proteins required for conjugation. One of these proteins forms the sex pilus.

- **Ribosomes:**

1) Ribosomes consist of two subunits, the smaller one is 30s and the larger one is 50s. Together, they make the 70s bacterial ribosomes.
2) They are made of protein, ribosomal RNA.
3) These proteins are different from the eukaryotic ribosomes. Antibiotics can target and inhibit the protein synthesis of the bacteria's proteins without affecting the synthesis of ours (our proteins).

<u>Structures outside the cell wall:</u>

- **Capsule:**

A gelatinous layer covering the entire bacterium. It is composed of polysaccharide, except in the anthrax bacillus, which has a capsule of polymerized d-glutamic acid.

The sugar components of the polysaccharide vary from one species of bacteria to another and frequently determine the serologic type (serotype) within a species. (Virulent factor): It makes the bacteria more disease causing (pathogenic).

Importance of the capsule:

- Protection.
- It is a determinant of virulence of many bacteria.
- Specific identification.
- Capsular polysaccharides are used as the antigens in certain vaccines
- The capsule may play a role in the adherence of bacteria to human tissues.

❖ Flagella:

- Flagella move the bacteria in a process called **chemotaxis.**
- The long filament is composed of many subunits of a single protein, **flagellin.**
- Flagellated bacteria have a characteristic number and location of flagella: some bacteria have one, and others have many; in some, the flagella are located at one end, and in others, they are all over the outer surface.
- Flagella are medically important for two reasons:

(1) Some species of motile bacteria (e.g., E. coli and Proteus species) may play a role in pathogenesis.

(2) Some species of bacteria (e.g., Salmonella species) are identified with the use of specific antibodies against flagellar proteins.

❖ Pilus or Fimbria:

Are hair-like filaments that extend from the cell surface. They mediate the attachment of bacteria to specific receptors on the human cell surface. They are found around the bacteria and they are shorter and also thinner than flagella. Bacteria which have pili are more pathogenic (causing disease) than the bacteria without pili. They are composed of subunits of pilin, a protein arranged in helical strands.

- A specialized kind of pilus: sex pili. A sex pilus specializes in conjugation. Allows the two bacteria to make tube between each other to exchange genetic material **between the male (donor) and the female (recipient).**

❖ Glycocalyx:

Is also polysaccharide in nature, helps the bacteria attach to surfaces. Example: Streptococcus mutans, on the surface of teeth. This plays an important role in the formation of plaque (results in teeth decay).

❖ Mesosome:

Invagination of cell membrane.

The exact structure and function of mesosomes are not known. However, it has been suggested that mesosomes take part in respiration (increasing the surface area), and in cell wall formation during cell division (binary fission).

❖ **Spores:**

Spores' Characteristics:

1) They are produced by Fungi and some types of bacteria
2) Spores are microscopic.
3) Spores are unit of asexual production.
4) Endospores are resistant asexual spores that develop inside some bacteria cells whose primary **function** is to ensure the survival of a bacterium through periods of environmental stress.
5) Spores are metabolically inactive but contain DNA, ribosomes, and other essential components.
6) Spores are resistant to chemicals, unusual situations…etc.
7) High-intensity **ultraviolet** light is suspected to overcome such resistance and to **kill spores** efficiently.
8) Spores can germinate to form bacteria that can cause diseases.

Differences between a human cell and bacterial cell:

Human cell	**Bacterial cell**
Eukaryotic	Prokaryotic
Complex	Simple
True nucleus	No nucleus (nucleoid)
No plasmid	Plasmid
Linear chromosomes	Circular chromosome
80 S ribosome	70 S ribosome
No cell wall	Cell wall